AN OVERVIEW OF THE ROLE OF ALGAE IN THE TREATMENT OF ACID MINE DRAINAGE[1]

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Abstract.--Preliminary studies utilizing the digestion and ashing of freshwater algae of several phyla, samples from acid mine drainage (AMD) sources, indicate relatively high concentrations of iron and manganese associated with the algae. A wetland treatment system was constructed in September 1987 at an abandoned drift mine in Pennsylvania, associated with the Lower Clarion Coal Seam. In this system, specifically designed and planted "algae ponds" are integrated with emergent marsh sections of the system in an effort to examine the role of algae in treating AMD. The site of mineral accumulation in the algae (adsorbed and/or intracellular concentrations) and the form(s) in which the minerals are found in the algae are of primary interest. The relationship between iron and manganese regarding uptake in the cells, the life histories and possible succession of predominant algae species in the ponds, the correlation between mineral uptake and water quality, the effects of mineral toxicity to the algae, and the potential ability of algae to modulate effluent pH values on a diel basis will also be investigated. An overview of methods and their rationale along with results to date at the noted study site will be presented, with the intent of allowing for the replication and expansion of the study by others.

INTRODUCTION

Freshwater algae are common and widespread inhabitants of the waters associated with coal mine drainage and are recognized as being effective accumulators of metals (Bates et al. 1982, Crist et al. 1981, Wetzel 1975). The majority of work related to algae and the bioaccumulation of metals has typically been with metals that are generally of little concern, from a regulatory standpoint, in coal mine drainage, notably zinc, nickel, copper, and lead.

Iron and manganese, however, are probably the two most common, and regulated, mineral constituents of mine drainage, accounting for millions of dollars in chemical treatment costs annually. Iron, which is relatively easy to remove from mine drainage, presents few logistical problems to chemical treatment, but manganese is a more persistent mineral and requires rather sophisticated treatment to achieve effluent standards. Additionally, concentrations of 50-100 mg/L of manganese are not uncommon in acidic mine drainage (Patterson 1975).

Algae require iron and manganese as essential micronutrients. Although concentrations of manganese > 1 mg/L have been shown to be inhibitory to some blue-green and green algae (Gerloff and Skoog 1957, Patrick et al. 1969), algae are commonly found and often abundant in AMD with high concentrations of iron and manganese. Algae associated with these areas have been shown to accumulate manganese, with reported levels of manganese as high as 56,000 mg/kg of plant tissue

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[1] Paper presented at the 1988 Mine Drainage and Surface Mine Reclamation Conference sponsored by the American Society for Surface Mining and Reclamation and the U.S. Department of the Interior (Bureau of Mines and Office of Surface Mining Reclamation and Enforcement), April 17-22, 1988, Pittsburgh, PA.

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Proceedings America Society of Mining and Reclamation, 1987 pp 286-290

DOI: 10.21000/JASMR88010286

http://doi.org/10.21000/JASMR88010286

(dry weight) (Kepler 1986). This is of particular interest in that biological treatment systems, i.e., man-made wetlands, are proving to be a viable and cost-effective means of treating AMD.

To date, most man-made wetlands are constructed as *Sphagnum*-dominated, or more commonly, *Typha*-dominated treatment systems (Kleinmann and Girts 1986). Algae are generally not purposefully introduced into these wetlands, but become established in the systems by way of the source of the mine drainage, the air, soil, and most commonly, in association with the principal, transplanted wetland vegetation. Because of this secondary nature of establishment, any beneficial effects of algae on the quality of AMD often go unrecognized, or the beneficial effects are limited because the Wetland Treatment System (WTS) is not designed to optimize algal habitat and therefore, algal biomass is limited. Little is actually known concerning the role algae has in the treatment of AMD in these Wetland Treatment Systems.

This paper is intended to be an overview of work in progress concerning the role of algae in the treatment of AMD in WTS's, with particular emphasis placed on the mechanisms involved in the bioaccumulation of manganese, Construction of the study WTS began in September of 1987, and therefore, limited findings are available at this time. However, the objectives and methods of the study, along with any results to date, will be reported and discussed.

DESCRIPTION OF RESEARCH

Background

This study is being funded by the United States Department of the Interior, Bureau of Mines, under the Abandoned Mine Land research program and is listed as, "Acid Mine Drainage Treatment Utilizing Blue-Green Algae." The study wetland is located in Richland Township, Venango County, PA, and was constructed in cooperation with Glacial Minerals, Inc., of Strattenville, PA, on land owned and previously surface mined by Glacial Minerals, Inc. The source of the acid mine drainage being treated by the WTS is from an abandoned drift mine (circa 1920) that has been discharging since at least 1940 and is now considered to be receiving recharge waters from the adjoining surface mine. The water is associated with the Lower Clarion Coal Seam and flows at an average of 37 gal/min. The AMD quality at the source is characterized as follows: pH, 5.0; alkalinity, 16 mg/L; mineral acidity, 450 mg/L; total iron, 130 mg/L; manganese, 71 mg/L; aluminum, 4 mg/L; and sulfate, 2,200 mg/L.

Construction

Construction began in September of 1987 with the majority of the system completed by November of that year. The WTS was designed and built with an initial collection pond immediately adjacent to the drift mine source, followed by two, distinct, parallel wetlands. The two wetlands combine at their respective outflow points and flow through a planted ditch to a conventional treatment pond series.

The parallel wetlands are set up as four individual units in each system, two shallow (6-12 in), *Typha*-dominated ponds followed by two, deeper (3-4 ft) algae-dominated ponds in one series; and two algae ponds followed by two *Typha* ponds in the other series. Each pond has an approximate surface area of 600 ft^2 and is connected to the next pond in series by a section of 6-in diameter plastic pipe. The system is graphically displayed in figure 1.

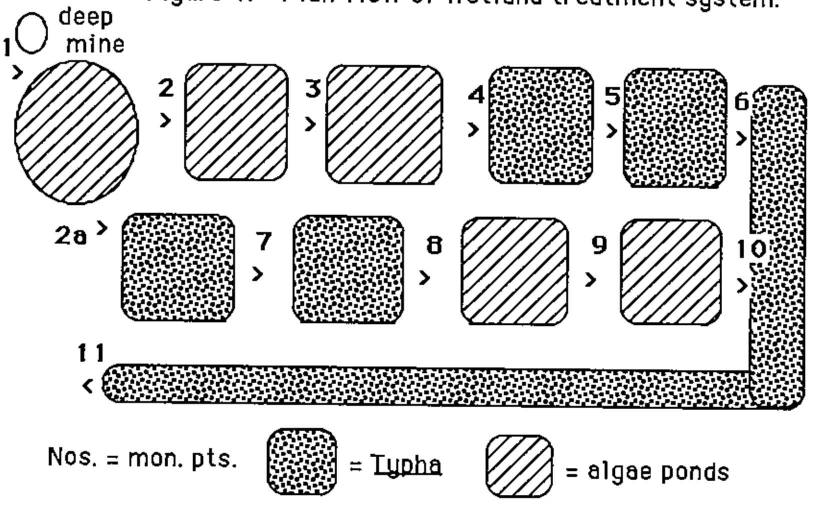
The ponds were constructed with available, on-site clay and a 12-in base of composted manure which serves as a planting medium and a source of nutrients to the vegetation. Four to six inches of agricultural limestone was placed beneath the compost in the final, planted ditch, but no alkaline materials were used in any other portion of the wetland. *Typha latifola* was available on site, and was hand dug and planted at a density of roughly one mature plant and rhizome mass per 2 ft^2. *Scirpus cyperinus* and *Juncus effusus* were also available on site and were introduced in the *Typha* ponds primarily in the areas of the inflow/outflow pipes. *S cyperinus* and *J. effusus* were also planted around the edges of the algae ponds to stabilize the banks and to provide possible sites of attachment for algae.

Initially, the study intended to concentrate on blue-green algae (Cyanobacteria) of the genus *Oscillatoria*. *Oscillatoria spp.* was noted as being prevalent in several natural and man-made wetlands that displayed reductions in manganese levels from source to outflow, and was the species noted above exhibiting manganese levels of 56,000 mg/kg of plant. However, during the period between the original proposal and the actual planting of the study wetland, the predominant algae in the previously *Oscillatoria*-dominated wetlands became the green algae of the genus *Mougeotia*. *Oscillatoria* was still present, but not dominant.

The water quality in these wetlands continued to show manganese reductions, and analysis of *Mougeotia* samples confirmed that they too were accumulating manganese. Therefore, the decision was made to transplant algae from one of these sources into the study WTS, knowing that the predominant algae being "planted" was *Mougeotia*, but that *Oscillatoria* was also being introduced at the study site. Approximately one 30-gal container of *Mougeotia*-dominated algae was introduced into each of the four algae ponds at the study wetland. Because the side of the parallel series with the *Typha* ponds down-flow of the algae ponds would be immediately affected by this planting and the other side would not be affected, a container of algae was also introduced into each of the four *Typha* ponds.

Various other species of algae, primarily diatoms, were also introduced into the wetland with the principal algae planting, and a filamentous green algae was noted as being associated with the *Typha* planting. These species, while of little apparent significance regarding total numbers at the time of the planting, are being identified and may be found to take on some degree of importance in treatment during the 1988 growing season.

Figure 1.--Plan view of wetland treatment system.



Water Analysis

The AMD source, the inflow/outflow for each pond, and the final outflow point of the wetland have been designated as water monitoring points in this study. Each monitoring point will be sampled weekly over the 18-month study which began in September of 1987. The samples are analyzed for pH (electrometrically); specific conductance (μ mhos at 25° C); alkalinity and mineral acidity (titrimetrically at pH 4.5 and 8.3 respectively); and total and ferrous iron, total and dissolved (dissolved: passing a 45-μ membrane filter) manganese, and sulfate and aluminum (all minerals are determined spectrophotometrically). Water temperatures (° C) are taken from each pond in the field at the time of collection. All analyses are conducted following the Standard Methods for the Examination of Water and Wastewater and are done in accordance with E.P.A. guidelines.

Flows are measured weekly by means of a portable cutthroat flume in the channels and by bucket and stopwatch at the discharge pipes. In this manner, the total water budget of the WTS may be known, with any increases or decreases in the system readily noted.

Vegetation Analyses

Representative samples of the wetland vegetation will be collected and analyzed for iron, manganese, and aluminum content beginning in the spring of 1988 and then periodically throughout the study, with principal concern given to the predominant algae species. Total metals are determined as follows: the samples are dried, ashed by means of heating at 550° C in a muffle furnace for two hours, acid digested by standard methods, and analyzed for mineral concentrations by atomic absorption. Results are reported as mg of mineral/kg of plant (dry Weight).

To determine the site of mineral accumulation in the algae, i.e., adsorbed and intracellular concentrations, the algae samples are "washed" with an EDTA solution in a shaker bath and then filtered. The extract is analyzed by atomic absorption to determine the concentration of adsorbed mineral associated with the algae. The "washed" algae is then ashed and analyzed as described above to determine the intracellular concentration of minerals.

The form of the minerals in and on the algae, e.g., adsorbed manganic oxide, will also be determined utilizing a form of x-ray diffraction so as to predict the long-term stability of the minerals in the wetland and the mechanisms of accumulation.

Diurnal studies will be conducted during periods of accelerated algal growth to examine the dissolved oxygen content and pH of the ponds and, therefore, whether the algal blooms have any effect on pH values. Carbon dioxide removal and production in the wetland, through algal photosynthesis and respiration, can account for shifts in pH values, although this phenomenon is generally associated with alkaline waters (Cole 1979). Lake pH's in western Pennsylvania have been noted to fluctuate five pH units over a 24-hr period due to the effects of blue-green algal blooms (Kepler 1985).

RESULTS AND DISCUSSION

Water

At the time of this writing, water quality data are available for the last two weeks of September 1987, through the first two weeks of January 1988. The numbers reflect the effects of a newly planted, somewhat dormant wetland. The Typha planting died back early in September simply because of the season, and the algae populations have not had favorable weather conditions to bloom. However, between the compost and the dead vegetation there is considerable organic matter present in the WTS, the rhizomes in the Typha ponds are active, bacteria and algae are present in the system, and the WTS is designed to maximize plant contact and retention time with the flows. Therefore, even at this early date, positive water quality results can be seen (figures 2-5).

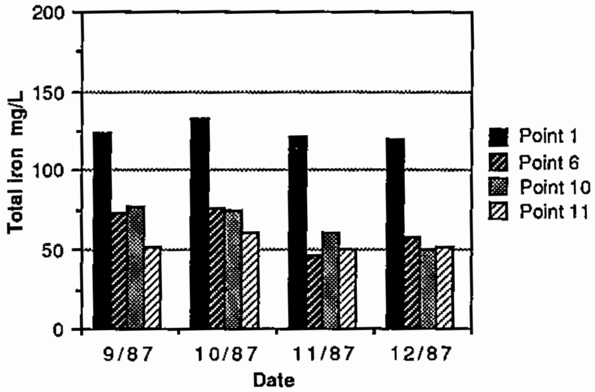


Figure 2.--Average total iron at AMD source, points 6 & 10, and final outflow.

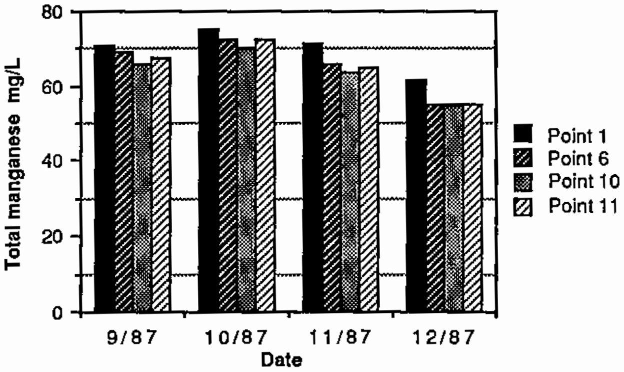


Figure 3.--Average total manganese at AMD source, points 6 & 10, and final outflow.

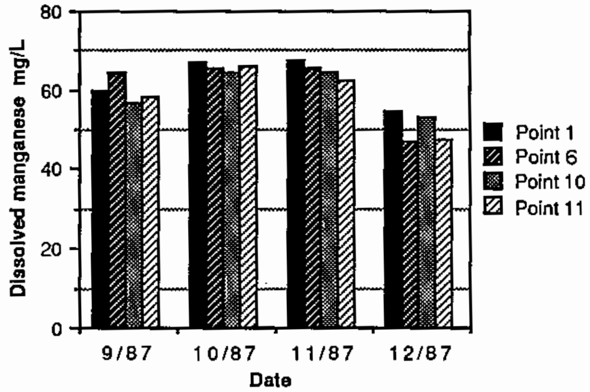


Figure 4.--Average dissolved manganese at AMD source, points 6 & 10, and final outflow.

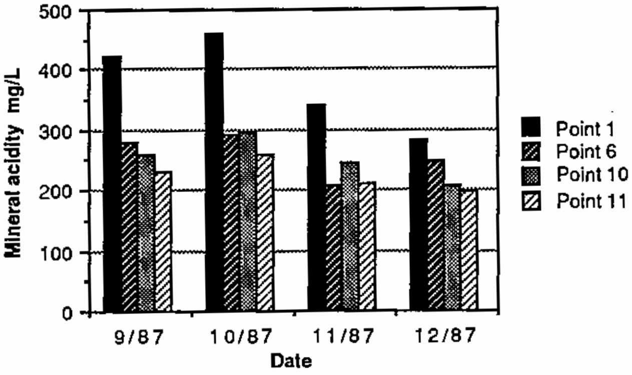


Figure 5.--Average mineral acidity at AMD source, points 6 & 10, and final outflow.

The WTS is significantly reducing the iron and acidity load of the AMD, but manganese values are not significantly reduced at this time. To date, the quantity of water entering and exiting the system has been equal. A small amount of seepage has been noted along the coal outcrop, upslope and immediately adjacent to the pond series ending with the *Typha* ponds (point 6), but the seepage is small enough that neither the quantity or quality of the flows in the WTS has been affected. The outcrop area has been planted in an attempt to control the seepage, since any drainage entering the WTS near its middle or end would obviously bias any results or conclusions regarding the effectiveness of the WTS.

Vegetation

The actual vegetative analyses will not begin until spring 1988 unless the algae ponds bloom during the winter months. The operational definition for "adsorbed minerals," in regard to the algae, is "that portion of the total minerals which can be extracted with the EDTA wash." The washing and filtration of the samples undoubtedly stresses the algae and may therefore bias the findings in relation to the concentration of adsorbed minerals. However, a similar study utilizing an EDTA wash to extract adsorbed zinc from algal samples indicated that the technique had no appreciable effect on the total concentration of surface-bound zinc, in that measurable intercellular zinc was not released by the technique (Bates, et al. 1982).

The most efficient method of mineral extraction for this study will be determined during the noted winter refinement period by testing varying lengths of extraction time, EDTA concentrations, and method of supernatant collection. The definition of adsorbed minerals will remain operational, however, since it will be impossible to unequivocally state that no intercellular minerals were released, and therefore measured, during the extraction process.

The ability to distinguish adsorbed from intracellular minerals will allow for a more complete understanding of the role of algae and the mechanisms of mineral accumulation in WTS's. Algal cell walls are composed of numerous, potential mineral adsorption sites as a result of their chemical makeup; however, these sites may or may not be active in transporting iron and manganese within the cell. The cell walls can act as both chemical and physical barriers in regulating mineral transport within the cell, thereby providing a means of metal tolerance to the algae by excluding potentially toxic ions from entering the cell (Foster 1977).

It is likely that the ashing data will show the majority of iron and manganese will be adsorbed versus intracellular; this is chiefly because of the known large concentrations of these minerals associated with the algae in the background ashings and the minerals' potential toxicity at these levels to the living cells.

Appropriate statistical analyses will be employed to determine whether intracellular concentrations of minerals are a function of the adsorbed concentrations, or if they are transported within the cell independently of the surface concentrations. Previous data also suggest that there may be an inverse relationship between iron and manganese accumulation in wetland vegetation (Kepler 1986), a relationship that will be tested in this study. The data will be further analyzed to determine if there is a correlation between total mineral concentrations in the AMD and total mineral uptake and accumulation in the algae; i.e., whether algae are more effective bioaccumulators at particular AMD mineral concentrations. Add to these findings the benefit of knowing the chemical form of the accumulated minerals, and predictions as to the success and long-term stability of WTS's will become more accurate.

SUMMARY

The removal of manganese in WTS's integrated with specific algae "ponds" may prove to be a successful treatment technique. Algae appear to be particularly well suited to this type of system in that they are widespread in AMD, are capable of accumulating relatively high concentrations of minerals, and are capable of rapid reproduction. The success of this single WTS and the mechanisms of the bioaccumulation of AMD minerals in algae will be determined in the proceeding months.

LITERATURE CITED

Bates, S. S., A. Tessier, G. C. Campbell, and J. Buffle. 1982. Zinc adsorption and transport by _Chlamydomonas variabilis_ and _Scenedesmus subspicatus_ (Chlorophyceae) grown in semicontinuous culture. J. Phycol. 18:521-529 http://dx.doi.org/10.1111/j.1529-8817.1982.tb03218.x

Cole, G. A. (Ed.). 1979. Textbook of Limnology. 426 pp. C. V. Mosby Company, St. Louis, MO.

Crist, R. H., K. Oberhosler, N. Shank, and R. Nguyen. 1981. Nature of bonding between metallic ions and algal cell walls. Environ. Sci. Technol. 15:1212-7. http://dx.doi.org/10.1021/es00092a010

Foster, P. L. 1977. Copper exclusion as a mechanism of heavy metal tolerance in green alga. Nature (Lond.) 269:322-3. http://dx.doi.org/10.1038/269322a0

Gerloff, G. C., and F. Skoog. 1957. Availability of iron and manganese in southern Wisconsin lakes for the growth of _Microcystis aeruginosa_. Ecology. 38:551-556. http://dx.doi.org/10.2307/1943118

Kepler, D. A. 1985. Unpublished data from observations of natural and man-made wetlands in western Pennsylvania.

_______. 1986. Manganese removal from mine drainage by artificial wetland construction. In: Proceedings of the 8th annual national abandoned mine lands conference, pp. 74-80. Billings, MT.

Kleinmann, R. L. P., and M. A. Girts. 1986. Constructed wetlands for treatment of mine water--successes and failures. In: Proceedings of the 8th annual abandoned mine lands conference, pp. 67-73. Billings, MT.

Patrick, R., B. Crumb, and J. Coles. 1969. Temperature and manganese as determining factors in the presence of diatom or blue-green algal floras in streams. Proc. Nat. Acad. Sci. 64:472-478 http://dx.doi.org/10.1073/pnas.64.2.472

Patterson, J. W. (Ed.). 1975. Wastewater treatment technology. 265 pp. Ann Arbor Science, Ann Arbor, MI.

Wetzel, R. G. (Ed.). 1975. Limnology. 743 pp. Saunders College Publishing, Philadelphia, PA.